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KINETICS OF THE NITROSAMINE FORMATION IN TOBACCO SMOKE
Formation of N-nitroso nornicotine from nornicotine and nicotine in
model trials

Summary

The formation of N-nitroso nornicotine has been studied from nornicotine and nicotine as free bases in gaseous mixtures comparable to cigarette smoke as to concentrations of the reactants.

Nornicotine as a free base - not protected as salt and retarded by intersolubility and competition with other smoke constituents, as it is in original cigarette smoke - is nitrosated to about 20% in 5 minutes. The time available for this reaction will be very much shorter in fresh natural smoke.

Of nicotine, as free base and purified from nornicotine, after 15 minutes at room temperature only 0,86% had reacted to form N-nitroso nornicotine. During a smoke puff followed by in- and exhalation the rate will be smaller by orders of magnitude. At 80°C and 220°C no detectable amounts of N-nitroso nornicotine have been formed. This is obviously due to the negative temperature coefficient of the oxidation constant of nitric oxide and the almost complete cleavage of dinitrogen trioxide (N_2O_3), the nitrosating agent, at those temperatures.

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Like many other tertiary amines nicotine is nitrosated in aqueous solutions but in the order to promille during several hours. Obviously two p_H maxima at about p_H 4 and p_H 1 appear.

The contribution of nicotine to the N-nitroso nornicotine content of cigarette smoke appears to be very doubtful. Only very small amounts of N-nitroso nornicotine may be formed due to the nitrosation of nornicotine under realistic conditions in the smoke.

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Summary of preceding progress reports

The chemiluminescence determination of nitric oxide in smoke puffs of 40 brands revealed the average content to be of the order of 400 vpm (540 ng/ml) nitric oxide, corresponding to 450 µg/cigt. (8 puffs). Almost no nitrogen dioxide is present in the genuine smoke.

Nitric oxide in model systems as well as in ageing smoke is oxidized to nitrogen dioxide in a pseudo-second order reaction with a half life time of 930 seconds in concentrations and under conditions corresponding to normal smoke.

In model trials nitric oxide is consumed much faster (exactly double the rate) in presence of secondary, and, to a lesser degree, by tertiary, primary amines, and ammonia, but much less it is diminished by alcohols. This enhanced decrease of the nitric oxide concentration is due to the nitrosating reaction:
$$\text{NO} + \text{NO}_2 + 2 \text{R}_2\text{NH} = 2 \text{R}_2\text{N-NO} + \text{H}_2\text{O}, \text{N}_2\text{O}_3 \text{ being the nitrosating intermediate.}$$

In ageing cigarette smoke the declining rate of nitric oxide was found to be of the same order of magnitude as with secondary amines.

Three factors relevant in the smokers respiratory tract strongly influence the half life time of the oxidation of nitric oxide, and probably, as a consequence, the formation of nitrosamines during smoking: 1. Elevated temperature (38°C), for the temperature coefficient for the necessary oxidation of nitric oxide is negative, 2. almost 100 % relative humidity reduce the oxidation rate and, 3. the oxygen content of the smoke-air mixture in the pulmonary alveoli only slightly exceeds the smoke oxygen content because of the competing absorption of oxygen by hemoglobin.

The importance of these factors has revealed from model trials in systems similar to smoke with respect to the concentration by means of the chemiluminescence methods for nitric oxide as well as for nitrosamines.

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In contrary to assumptions discussed recently, nitric oxide is not quantitatively retained in the smokers respiratory tract. The exhalate of the first breath after inhalation with three smokers accounted for between 15 and 45 % of the nitric oxide of a puff.

Very specific measurements of nitrosamines can be achieved by the determination of the nitric oxide formed by thermal decomposition of the nitrosamines and application of the sensitive and selective chemiluminescence method under investigation. If special precautions are observed, this determination can be applied even in presence of oxygen, i.e. to tobacco smoke.

In order to pursue this aim the method has been applied to the effluents of a simple and special gas chromatographic set up, which shall be applied to smoke.

Different trapping systems usually applied to nitrosamine determinations in tobacco smoke have been shown by this means to give rise to remarkably different results.

Comparison with data collected from preceding studies on the reaction kinetics and by means of experimentally determined reaction constants of the gas phase reactions involved in the formation of volatile nitrosamines revealed some traps to deliver too small figures, probably due to escaping - alkali and other aqueous traps, - whereas others were shown to cause much higher values than to be expected from kinetic data (solvents/dry ice).

The formation of N-nitroso nornicotine from nornicotine in aqueous solutions had been found to be the highest at p_H 3.8. At p_H 1.5 and lower and at p_H 5.2 and higher almost no reaction occurs.

In 1-molar buffers the reaction rate was found to be smaller than in 0.2-molar buffer solutions.

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Introduction

Papers of Klus and Kuhn (15) as well as Hoffmann, Rathkamp, and Liu (16) on the occurrence of N-nitroso nornicotine in cigarette smoke and a paper of Hoffmann, Hecht, Ornaf, and Wynder (17), who reported the occurrence of N-nitroso nornicotine in unburned tobacco also, have focussed the interest to the formation of that nitrosamine not only from nornicotine but also to that from nicotine. This latter in context with Lijinski's publications dealing generally with the formation of nitrosamines from tertiary bases (18).

This report deals with part of the reaction kinetic studies on the formation of N-nitroso nornicotine (NNN) from nicotine and nornicotine in aqueous solutions and gas phases comparable to cigarette smoke as to concentration of the respective reactants and conditions.

Nitrosation of nornicotine and nicotine in gaseous mixtures by nitric oxide and oxygen.

In order to approach the conditions of nitrosating possible in the tobacco smoke aerosol, similar systems have been applied as already described in Progress Reports 2 and 3.

Two four-necked flasks of 540 ml each, connected with a teflon tube, were evacuated to about 0.1 mm/Hg. The first was filled with nitric oxide test gas of 1000 vpm nitric oxide in nitrogen. Into the second 10 μ l nornicotine or 50 μ l nicotine (nornicotine content less than 10 ppm) were injected through a septum. The flasks have been opened to each other and through the second flask air has been let in so, that the whole system has been adjusted to atmospheric pressure and to an oxygen concentration of about 10 %, corresponding to cigarette smoke.

Nornicotine and nicotine as free bases participated in the reaction in concentrations according to their vapor pressures at the respective temperatures: 22°C and 80°C. I.e., nitric oxide had been in excess at 22°C, whereas the tobacco alkaloids had been in excess at 80°C.

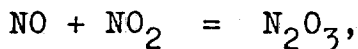
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The reactions have been pursued by gas chromatographic determination of N-nitroso nornicotine (NNN) after isolation by extraction of the gas mixtures with 10 ml dichloromethane at the listed time intervals. For each measurement a new run had been necessary this way. Completeness of the extraction of NNN has been proved by sequential extraction, which revealed no further NNN to be present in the extracted systems.

The results are summarized in tables 1 and 2 showing, that nornicotine, as free base and not hindered by other components by intersolubility or competition for N_2O_3 , i.e. under optimal conditions for the gas phase reaction as to salt/free base-proportion and access of the free base to the gas phase, reacts fairly rapidly like other secondary bases.

Nicotine, however, also forms NNN, but after 15 minutes less than 1 % had been nitrosated at $22^{\circ}C$.

At $80^{\circ}C$ and $220^{\circ}C$ no formation of NNN from nicotine occurs, which seems to be due to the fact, that according to the negative temperature coefficient of the oxidation of nitric oxide almost no nitrogen dioxide can be formed. Furthermore under these conditions, the amount of NO_2 formed, does not with the abundant NO combine to N_2O_3 , the nitrosating agent, because the equilibrium



at those temperatures, is shifted extremely to the left side of cleavage. At $100^{\circ}C$ for instance and 0.01 atm. only 0.03 % appear in the form of dinitrogen trioxide; the partial pressure of NO in tobacco smoke, however, is only 0.0005 atm.

In this light the formation of NNN from nicotine in cigarette smoke appears hardly to be a realistic contribution to the nitrosamine content of the smoke facing the short time consumed by puff, in- and exhalation of smokers.

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Table 1

N-Nitroso nornicotine formation from nornicotine in gaseous mixtures of oxidizing nitric oxide at 25°C

| time | µg NNN formed | run 1 | run 2 |
|----------|------------------|-------|-------|
| 5' | | 375 | 390 |
| 10' | | 490 | 502 |
| 15' | | 580 | 586 |
| 30' | | 686 | |
| 60' | | 765 | |
| 16 hours | | 997 | |

Table 2

N-Nitroso nornicotine formation from nicotine in gaseous mixtures of oxidizing nitric oxide at 25°C

| time | µg NNN formed | % of the nicotine which is nitrosated, calculated from vapor pressure |
|------|------------------|--|
| 15' | 4.7 | 0.86 |
| 30' | 9.5 | 1.73 |
| 60' | 15.8 | 2.88 |

at 80°C and 220°C

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Nitrosation velocity of nicotine in aqueous solutions at different p_H values

For comparison with the results on the nitrosation velocity of nornicotine (see Progress Report No. 4) a similar study was accomplished to the nitrosation reaction of nicotine in aqueous solutions.

Hoffmann, Rathkamp, and Liu (16) reported yields of 1 - 7 % after one hour depending on the p_H value. But the authors did not give data about the purity of the nicotine involved in their study.

In order to avoid interferences with natural nornicotine almost ever present in not specially purified samples of natural nicotine, the raw nicotine was freed from nornicotine by nitrosation and separation of the latter by distillation.

The purity of the resulting nicotine, controlled by gas chromatography was 99.9 %; the final impurity by nornicotine was less than 10 ppm.

Gas chromatographic measurement of the N-nitroso nornicotine (NNN) formed in aqueous solutions at p_H values, actually measured and differing from 1.7 to 6.6, at 22°C, revealed, that after one hour only at p_H 1.7 and p_H 2.5 0.32 and 0.20 promille respectively of the nicotine had been nitrosated. The highest amounts measured in the series were obtained after 8 days at p_H 4.0 - 5.0 with 3.3 promille NNN (see table 3). At longer reaction times, from 21 hours on, there seem to be two p_H maxima corresponding to two different ways of reaction, but this was not pursued so far.

At 80°C the reaction proceeds faster. At p_H 3.8 after 5 minutes already more than 1 % of the nicotine had reacted, the highest values observed being reached after 2 hours with 8.49 % NNN formed from nicotine (see table 4).

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Table 3

N-Nitroso normicotine (promille of nicotine reacted)
at 22°C at different p_H values

| time | pH | 1.7 | 2.5 | 4.0 | 4.2 | 5.0 | 6.6 |
|---------|----|------|------|-------|-------|-------|-------|
| 30' | | 0.21 | 0.10 | ∅ | ∅ | ∅ | ∅ |
| 60' | | 0.32 | 0.20 | ∅ | ∅ | ∅ | ∅ |
| 2 h | | 0.81 | 0.28 | trace | trace | trace | ∅ |
| 4 h | | 0.87 | 0.46 | trace | trace | trace | ∅ |
| 21 h | | 1.13 | 0.49 | 1.4 | 1.4 | 1.0 | trace |
| 28 h | | -- | -- | 2.1 | 1.7 | 1.1 | - |
| 2 days | | 1.21 | 0.49 | 2.2 | 1.6 | 1.8 | trace |
| 3 days | | -- | -- | 2.3 | 2.3 | 2.3 | - |
| 4 days | | 1.38 | 0.48 | - | - | - | 0.21 |
| 8 days | | 1.41 | 0.51 | 3.3 | 3.3 | 3.3 | 0.32 |
| 13 days | | -- | -- | 3.0 | 3.0 | 3.0 | -- |

Table 4

N-Nitroso normicotine (% of nicotine reacted) at 80°C at p_H 3.82

| time | p _H 3.82 |
|------|---------------------|
| 5' | 1.23 |
| 15' | 3.15 |
| 30' | 4.88 |
| 45' | 6.06 |
| 60' | 6.42 |
| 100' | 7.81 |
| 120' | 8.49 |
| 150' | 8.49 |
| 180' | 8.49 |
| 210' | 8.49 |
| 300' | 8.49 |
| 48 h | 6.0 |

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Nitrosation conditions in aqueous solutions

About 9 ml of the respective buffer solutions (0.2 molar) 100 μ l specially purified nicotine, and 0.5 ml 1n-sodium nitrite solution were stirred at 22°C or 80°C.

The actual p_H values of the reaction solutions were measured by means of an electrometric p_H -meter equipped with a glass electrode.

At time intervals, given in tables 3 and 4, 0.5 ml samples of the solution were taken, basified with aqueous potassium carbonate solution and extracted with 100 μ l dichloromethane.

2 μ l of the dichloromethane solution were injected for the gas chromatographic determination.

This extraction procedure was compared to the extraction at the respective p_H values of the reaction solution. No difference was found due to the p_H value of the extracted solution.

Gas chromatographic procedure:

Device: Hewlett-Packard hp 5751 G. 1.50 m glass beads, washed with 1 % Silar 5CP in chloroform.

Column: temperature program 70-120°C, 4°C/min, injection port 270°C, FID 330°C, 30 ml nitrogen/min, 30 ml hydrogen/min, 500 ml air/min.

Nicotine purification

15 g nicotine dissolved in 1 n hydrochloric acid were brought to p_H 3.65 by adding a buffer solution. About 2 g sodium nitrite were added and the solution stirred for 36 hours. After acidifying with 10 % hydrochloric acid, the mixture was extracted three times with dichloromethane. The remainder was basified with 50 % potassium hydroxide solution and extracted with ether. After removing of the ether the nicotine was purified by distillation in vacuum: Kp_{15} 119°C.

No detectable traces of nornicotine appeared in the gas fractogram.

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Literature

- (15) H. Klus and H. Kuhn
Fachl. Mitt. österr. Tabakregie 14 (1973) 251-257
- (16) D. Hoffmann, G. Rathkamp, and Y.Y. Liu
in P. Bogovski and E.A. Walker
N-Nitroso Compounds in the Environment, IARC
Scientific Publication No. 9, Lyon (1975) 159-165
- (17) D. Hoffmann, S.S. Hecht, R.M. Ornaf, and E.L. Wynder
Science 186 (1974) 265-267
- (18) W. Lijinski and G.M. Singer
in P. Bogovski and E.A. Walker
N-Nitroso Compounds in the Environment, IARC
Scientific Publication No. 9, Lyon (1975) 11-114

Literature is numbered through all progress reports on
"Kinetics of the Nitrosamine Formation in Tobacco Smoke"

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